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BIONOMICS OF THE LYMNAEID SNAIL, STAGNICOLA BULIMOIDES TECHELLA, THE INTERMEDIATE HOST OF THE LIVER FLUKE IN SOUTHERN TEXAS[1]

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INTRODUCTION

The liver fluke (*Fasciola hepatica* Linn.) is one of the most common and economically important parasites of cattle and sheep in the Gulf coast region of the United States. Slaughter records for 10 years under Federal meat inspection, obtained from six Gulf coast packing houses in Texas, Louisiana, and Florida, show 37.5 percent of the native adult cattle and 6 percent of the calves to be infected with the liver fluke. This condition results in the loss of the liver as an item of food, together with many unthrifty cattle and some death losses. Although the economic significance of this parasite and its general distribution in Texas were reported in 1891 by Francis (*4*),[2] it was not until about 40 years later that Sinitsin (*14*, *15*) demonstrated that *Stagnicola bulimoides techella* (Hald.), a common fresh-water snail, was the intermediate host of the liver fluke in the coastal region of Louisiana and Texas. A prerequisite in the planning of effective control measures against this snail is a knowledge of its bionomics.

REVIEW OF WORK OF PREVIOUS INVESTIGATORS

A study of the literature shows that the bionomics of the principal snail intermediate hosts of the liver fluke in different parts of the world depends on the species involved and the localities in which they are found.

In the British Isles, *Galba truncatula* (Müller), the common European intermediate host of the liver fluke, was found by Walton (*23*) to be active throughout the year, producing two generations annually during seasons of normal precipitation and three generations during wet periods. According to Peters (*10*), the snails showed a preference for regions having clay soils over which water moved slowly and were found rarely in streams, even during drought. Walton and Wright (*24*) found these snails in water having a pH of 6.0 to 8.6. They concluded that the hydrogen-ion concentration of the water was of little importance in determining the distribution of the snails.

In Germany, Mehl (*9*) classified the habitats of *Galba truncatula* into three types, depending on the prevalence of water in each. They were as follows: (1) Streams and springs, where frost, heat, and drought did not affect the snails and in which the water was rich in oxygen, thus maintaining large populations throughout the year

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[2] Italic numbers in parentheses refer to Literature Cited, p. 402.

under conditions favorable for continuous breeding; (2) roadside excavations and drainage and irrigation ditches that became dry during normal summers but might retain water during rainy years; and (3) meadows and flood plains on which rainfall, overflows, or irrigation provided favorable conditions for the snails. After droughts that destroyed the snail populations, high water and irrigation restocked these last-mentioned areas from the stream beds. The snails were observed to have a longevity of 10 to 17 months. They were capable of surviving desiccation as long as 4½ months, depending on temperature, soil type, available shade, depth to which the snails entered the soil, and the manner in which the shell orifice was closed.

In Australia, Ross and McKay (*11*) found *Limnea brazieri* (Smith), the intermediate host of *Fasciola hepatica*, in shallow pools, on mud, on sandy and rocky bottoms, in deep water, and clinging to stones in swiftly running rivers. *L. brazieri* was unable to withstand desiccation longer than 24 hours. Under natural conditions, longevity appeared to be about 12 months. Reproduction occurred, generally, during spring and summer although it was more or less continuous throughout the year but on a lower level.

In the Philippine Islands, *L. philippinensis* Nevill, the intermediate host of *Fasciola gigantica* Cobbold and *F. hepatica*, was destroyed in the field by a drought of 3 months' duration, even though the soil did not become dry immediately after cessation of rain, according to De Jesus and Mallari (*8*) and De Jesus (*7*). The snails lived as long as 94 to 110 days in the laboratory.

In the Hawaiian Islands, Alicata (*1*) found that *Fossaria ollula* (Gould), the intermediate host of *Fasciola gigantica*, bred throughout the year under natural conditions. The snails generally inhabited the shallow, lowland marshes but were found occasionally in swiftly flowing streams. Their longevity in the laboratory was 114 to 164 days. It was reported also by Alicata, Swanson, and Goo (*2*) that complete drainage would kill all snails and their eggs in about 5 days. The range of *F. ollula* includes both China and Japan, from which they were introduced into Hawaii.

In Puerto Rico, Van Volkenberg (*21*, *22*) found *Lymnaea cubensis* Pfr., the intermediate host of *Fasciola hepatica*, living in stagnant water of swampy regions, in shallow sluggish streams, and in drainage ditches but not in swiftly running water or on gravelly stream beds.

In Canada, *Fossaria parva* (Lea) and *Stagnicola palustris nuttalliana* (Lea), the intermediate hosts of *Fascioloides magna* (Bassi), were found by Swales (*16*) to have different environments. *Fossaria parva* preferred areas that were wet and swampy in spring but completely dry during summer and autumn. The snails were very active and were frequently found on the mud. Survival during drought apparently took place in the soil, where the young snails were found during July. *S. palustris nuttalliana*, on the other hand, preferred permanent or semipermanent bodies of stagnant water containing much vegetation. The snails remained in the water and did not move out on the mud. The alkalinity of the water appeared not to influence the distribution of the snails.

In the United States, Francis (*4*), as already indicated, was the first investigator to point out the economic importance of the liver fluke in Texas. He recognized the moist, low-lying coastal plain as

being the region of endemic fascioliasis, exceptions being the salt marshes where cattle were said to be relatively free of the parasite. However, he made no mention of the snail intermediate host in the region where he had conducted his investigations for a period of 3 years. He pointed out that fascioliasis occurred in cattle only when they had access to stagnant ponds that contained cercariae or to grass grown in wet places. Many years after the report of Francis on the liver fluke, Sinitsin (*15*) showed *Galba bulimoides techella* to be the common intermediate host of the liver fluke in Texas. He found the snails living on the muddy borders of both natural and artificial bodies of water.

The first natural infections of North American snails with *Fasciola hepatica* were reported independently by Shaw and Simms (*12*), in Oregon, for *Lymnaea* (*Galba*) *bulimoides* Lea, and by Sinitsin (*14*), in Louisiana, for *Galba bulimoides techella* (Hald.). These investigators verified the correctness of their observations by feeding the metacercariae to experimental animals in which infections with *F. hepatica* were obtained. In Oregon, Shaw and Simms (*13*) found the snails only in fresh water, the principal habitats being springs, seepages, and the banks of small streams. The snails were found at altitudes from near sea level to 5,600 feet, where the temperature was known to reach −40° F.

Fossaria parva (Lea) was reported by Hoff (*5*, *6*) to produce but a single generation per year in Illinois, whereas Van Cleave (*19*, *20*) found that *F. modicella* produced two generations annually in Indiana. *F. modicella*, the intermediate host of the liver fluke in Utah, was found to be very resistant to freezing as it went into hibernation when the temperature fell to 40° F. (*17*, *18*).

In view of these differences in the bionomics of the snail intermediate hosts of the liver fluke, it was considered desirable to make a detailed study of the bionomics of *Stagnicola bulimoides techella* in southern Texas. The results are reported in the present paper.

SCOPE AND METHODS OF WORK

Field investigations were made in 1940 and 1941 in the Gulf coast prairie, which is the upper part of the Gulf coast region of Texas, to determine the principal types of habitats of the snails, to study their life history during the different seasons, and to obtain data on reproduction, survival, longevity, dissemination, and natural infection. The work was carried on during all the seasons and under conditions ranging from extreme drought to abnormally high precipitation. Laboratory studies were conducted simultaneously to verify some of the observations and also to provide information that could not be obtained readily in the natural habitat of the snails.

The life cycle and number of generations of the snails were determined under natural conditions by making frequent collections and observations in a series of habitats. The snails obtained from these places were measured and then divided into adult and juvenile classes based on average size at which maturity occurred, as predetermined in laboratory cultures. The percentage of juvenile snails found during the various months or seasons indicated the appearance of a new generation or the maturation of a previous one.

In the life-cycle studies in the laboratory, cultures of snails were kept in large, moist chamber dishes. The snails lived and reproduced most successfully when the bottoms of the dishes were covered with mud, part of which was exposed above the shallow water. Filamentous green algae provided the most suitable type of food.

Data on survival during drought were obtained in the field by collecting estivating snails from the dry soil of the habitats at different periods between the time the pools dried and the reappearance of the water. Supplementary data on this point were obtained in the laboratory by exposing snails to desiccation. The snails were separated from the soil by washing it through a series of screens. This method also gave data on the depth to which the snails had entered the soil at the time the pools dried.

Natural infection of the snails was determined (1), by placing in large battery jars specimens collected from pastures where infected cattle grazed to determine whether they were shedding cercariae, and (2), by crushing specimens and making microscopic examination for the intramolluscan stages.

CHARACTERISTICS OF THE GULF COAST PRAIRIE OF TEXAS

The Gulf coast prairie of Texas is 20 to 80 miles in width and, according to Carter (*3*), extends from the eastern border of the State "to the vicinity of the San Antonio River" in the west. It borders the Gulf of Mexico in the south, where it rises gradually from sea level to an elevation of about 100 feet in the northern part.

Geologically, this region is characterized by flat terrain consisting of heavy, clay soils covered with an abundant growth of coarse grasses and having poor drainage. It is traversed by a number of rivers, the broad flood plains of which form strips of red alluvial soil that constitute the rich, wooded bottom lands. According to Carter, the black prairie soil of the Lake Charles series is the most extensive type in the region. This soil is generally deep and rests on heavy, impervious clay subsoil. Numerous shallow depressions of natural origin known as hog wallows are characteristic of the black prairie soil.

As shown in figure 1, the climate of the Gulf coast prairie is mild, the average annual temperature being 68° to 70° F. The winters, which constitute the principal wet season, are both mild and brief, the periods of freezing temperature being few and of short duration. The summers, on the other hand, are long and hot and frequently have prolonged periods of little or no rainfall over large sections. The average annual rainfall is about 50 inches. The greatest precipitation generally occurs during July, September, and December and the lowest during January, February, March, and April (fig. 1). As shown also in figure 1, the average annual evaporation exceeds the average annual precipitation from the beginning of March until late in August, during which time the surface pools are usually dry. This period of dryness commonly extends into October. Rain occurs most frequently during midwinter and midsummer. The average relative humidity is about 80 percent.

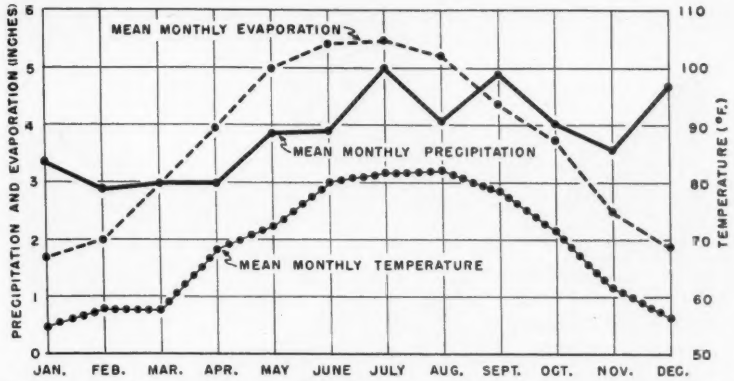

FIGURE 1.—Mean monthly precipitation, evaporation, and temperature in the Gulf coast prairie of Texas for the period 1914–41, as recorded at the Texas Agricultural Experiment Station, Substation 3, Angleton, Tex.

TYPES OF SNAIL HABITATS AND THEIR PHYSICAL CHARACTERISTICS

The habitats of *Stagnicola bulimoides techella* in the Gulf coast prairie are of two types—semipermanent ponds and temporary pools.

The semipermanent ponds retain water well into the summer and often throughout the year. They are represented by roadside excavations, sloughs (fig. 2, *A*), and well overflows. Because of the open water on one side and the dry bank on the other, both of which serve as barriers to the snails, the habitable region of the semipermanent pond is confined to the narrow margin of shallow water and the wet mud on the bank.

The temporary pools contain water only when precipitation exceeds evaporation sufficiently to produce an accumulation of surface water. The types of pools in this group are of both natural and artificial origin. Natural depressions include marshy areas, shallow drainage courses, and numerous other low places known as hog wallows (fig. 2, *B*), in which water collects. Artificial pools arise from any land use that leaves an uneven surface, the principal causes being excavations, worn pasture roads, cowpaths, hoofprints, wagon or implement tracks (fig. 2, *C*), and cultivated fields that are permitted to revert to pasture without being properly leveled. The temporary pools, with their sparse vegetation and extensive soil-water peripheries, provide a much more favorable environment for snails than do the semipermanent pools. This fact is demonstrated by the data in table 1. Semipermanent pools included in a study made in Brazoria County, Tex., during essentially the same period and on similar types of soil had a much higher percentage of juvenile snails than did the temporary pools, indicating that a large proportion of the snails had died before reaching maturity.

Marshy areas resulting from overflows of wells have the shallow water and exposed mud of the temporary pools and the duration of the semipermanent ponds.

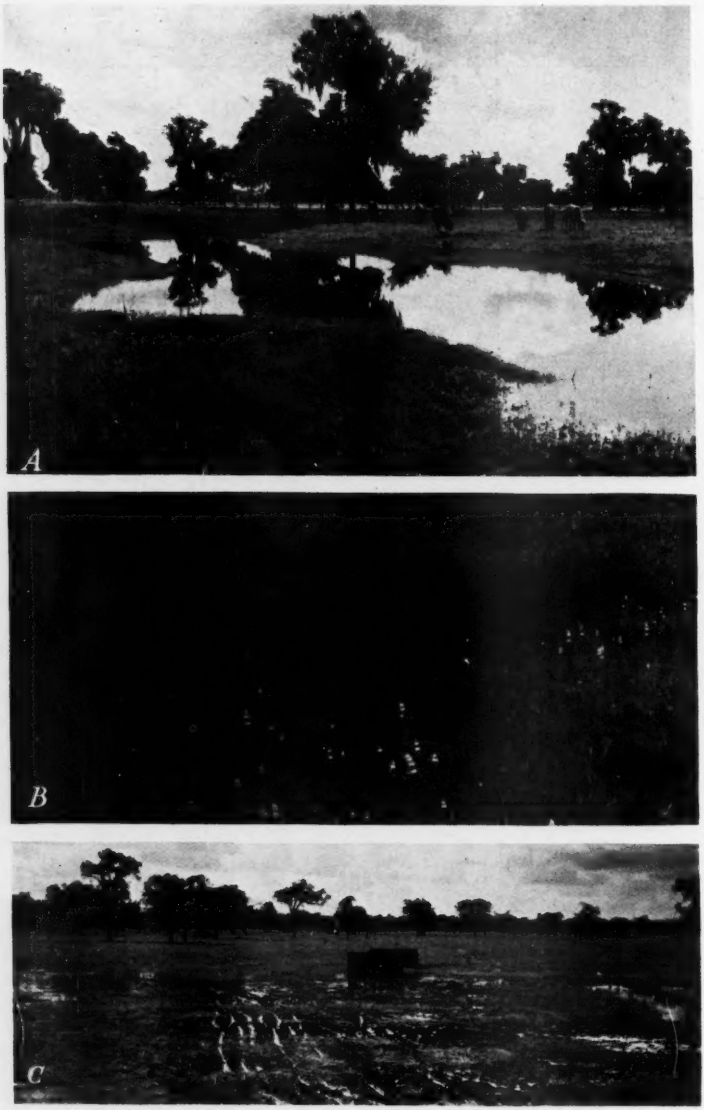

FIGURE 2.—Types of habitats of *Stagnicola bulimoides techella* in the Gulf coast prairie of Texas: *A*, Slough of semipermanent type in which the narrow, flat banks (lower right) support large numbers of snails, whereas the steep banks (upper right) are without snails. *B*, Shallow surface depressions known as hog wallows, indicated in the photograph by the numerous areas of darker vegetation, constitute a large portion of the area. *C*, Wagon tracks and other depressions in this bottom-land pasture increase its danger as a snail habitat during the wet season.

TABLE 1.—*Proportions of juvenile and adult* Stagnicola bulimoides techella *in semipermanent and temporary pools in Brazoria County, Tex.*

Semipermanent pools				Temporary pools			
Date of collection	Snails collected	Proportion of—		Date of collection	Snails collected	Proportion of—	
		Juvenile snails	Adult snails			Juvenile snails	Adult snails
1941:	*Number*	*Percent*	*Percent*	1941:	*Number*	*Percent*	*Percent*
July 15	221	62.7	37.3	July 30	163	28.8	71.2
July 31	550	69.3	30.7	Aug. 14	360	4.4	95.6
Aug. 18	400	57.8	42.2	Aug. 19	500	42.0	58.0
Sept. 17	720	98.5	1.5	Sept. 26	220	10.9	89.1
Sept. 26	336	83.6	16.4	Sept. 29	320	41.8	58.2

The two types of habitats have many physical similarities, particularly in type of soil and source of water. The pH of the water in the area studied ranged from 7.1 in certain well overflows to 8.4 in some of the temporary pools on alluvial soils. In general, temporary and semipermanent pools originating from rainwater were less alkaline on the black prairie soils than on the red alluvial soils. The hydrogen-ion content of the water appeared to have no influence on the distribution of the snails.

Temperatures in and around pools fluctuate greatly, depending on the season, time of day, amount and density of shade, and degree of exposure. Variations in temperature resulting from the last-mentioned factor are shown in table 2. The table also shows that during the summer months exceedingly high daytime temperatures prevailed in the shallow water and on the soil, whether wet or dry.

TABLE 2.—*Summer temperatures recorded in 2 types of habitats of Stagnicola bulimoides techella, in southern Texas under conditions of both drought and moisture*

Type of habitat	Date	Temperature of—			
		Air	Shallow water	Saturated mud at water's edge	Dry soil
		°F.	°F.	°F.	°F.
Low; well protected	June 26, 1940	91.4			146.3
	July 7, 1941	89.6	100.4	102.2	102.5
Flat; fully exposed	July 18, 1941	93.2	102.2	105.8	117.5
	Sept. 26, 1940	77			100.4

LIFE HISTORY

Egg masses of *Stagnicola bulimoides techella* are deposited most frequently on the wet mud at the water's edge or in the shallow water along the margin of the pool. In aquaria, egg masses have appeared more often on the exposed wet mud than in the water. Their size varies greatly, ranging from 2.5 to 12 mm. long by 1.8 to 4.2 mm. wide. The number of eggs counted in 36 masses ranged from 9 to 50 each with a mean of 22 eggs per mass.

Hatching of the eggs may occur as soon as 6 days after oviposition during the summer when the temperature is high. The young snails are about 0.6 mm. long when hatched and live on the saturated mud at the water's edge (fig. 3). Of the large numbers of eggs incubated in the laboratory, relatively few failed to hatch.

Growth of the young snails is very rapid when nutrition and moisture are favorable. Sexual maturity, which occurs when the shell reaches a length of about 4.5 mm., is attained as early as 14 days after hatching. Reproduction begins as soon as the snails

FIGURE 3.—Juvenile and adult snails on the wet mud and in the shallow water of a much-used and barren herd pen.

reach sexual maturity and may continue 3 to 7 months, depending on environmental conditions.

To determine the course of the life cycle of the snails in the field, including the number of generations and the time of the year when they occur, 102 collections in 40 localities, including more than 32,000 individuals, were made for a period of 2 years. The percentage of juveniles in the population for each month is shown in figure 4. As indicated by the figure, the June population is predominantly juvenile. Owing to the usual disappearance of the water from the habitats at this time, the young snails estivate in the soil during the period in which the pools are dry. Most of the old snails die without entering the soil. This fact was clearly demonstrated in a study of the snail population in one pool in southern Texas. A collection made on May 9, 1940, after the pool had dried, showed that most of the snails dead on the surface of the soil were adults (table 3). On October 30, after the water had reappeared in the pool, the surviving population was predominantly juveniles. Growth was rapid and

TABLE 3.—*Percentage of juvenile and adult Stagnicola bulimoides techella in the population (both living and dead) shortly before and immediately after estivation, which extended from May 9 to October 30, 1940, in a pool in southern Texas*

Date (1940)	Snails collected	Juveniles	Adults
	Number	*Percent*	*Percent*
Apr. 11	226	57.5	42.5
May 9	38	8.0	92.0
Oct. 30	942	98.4	1.6
Nov. 14	398	37.5	62.5

the population quickly passed from one of juveniles to one of adults, as shown by the percentages of each on November 14.

As indicated by the data in figure 4, obtained from extensive studies, mass oviposition takes place by the first of the year and the adult snails disappear for the most part. Thus, the longevity of the spring

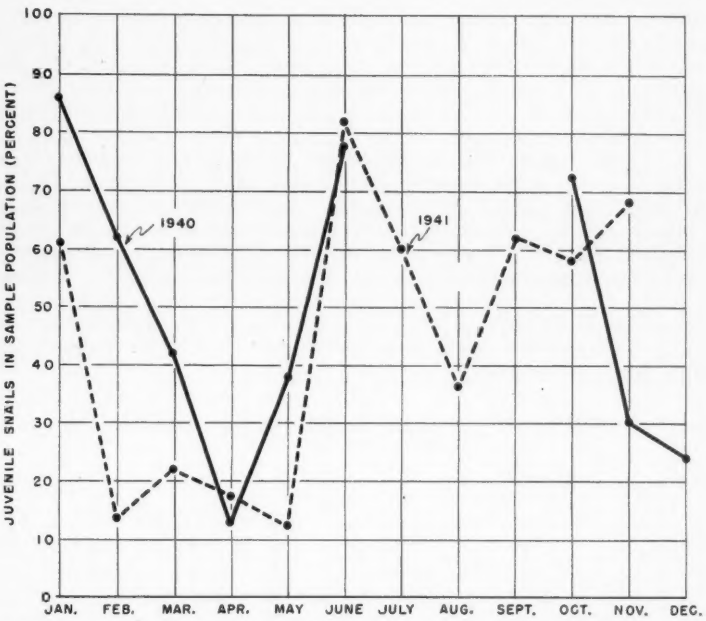

FIGURE 4.—Seasonal distribution of *Stagnicola bulimoides techella* in the Gulf coast prairie of Texas in 1940 and 1941. Pools were dry during July, August, and September 1940.

generation is about 6 months. The January population consists of a new generation of juvenile snails that originated from the June generation. These snails develop rapidly, oviposit, predominate in the population for a short time as adults, and then die, having lived about 6 months. Thus, under field conditions the general trend is

two distinct generations annually with some overlapping of reproduction. This condition exists whether the years be dry, as in 1940 when the pools were without water from June until the last of October, or very wet, as in 1941 when water persisted in them throughout the summer.

That the snails are capable of living much longer than the usual 6 months is shown by the fact that one individual survived in the laboratory for 25.5 months. This specimen was kept in a battery jar containing about 3 inches of mud to which water was added once monthly and permitted to remain 2 to 7 days, when it was drained off. The jar was kept in an open shed or in the laboratory, where it was protected from direct sunlight. When the soil began to dry, the snail burrowed into the mud where it remained until water was added. Both growth and reproduction occurred to a limited degree under these conditions.

EGG PRODUCTION

Since *Stagnicola bulimoides techella* is hermaphroditic, the entire population is capable of producing fertile eggs. This fact, together with the short juvenile stage and the long period of fecundity, accounts for the occurrence of a large population of snails in a habitat in a remarkably short time when the physical and nutritional conditions of the environment are favorable (fig. 3).

In the present studies, as shown in table 4, four laboratory-raised *S. bulimoides techella* began ovipositing 14 to 17 days after hatching and continued for 84 to 211 days, the average production being 5,112 eggs. Individual production ranged from 3,274 in 94 days to 6,479 in 129 days of laying time. The number of eggs was relatively low at first, rose to the maximum, and then decreased. A shortage of food in the aquaria resulted in a decrease in the number of eggs. The addition of food was reflected in a return to the previous number. Under favorable circumstances, the period of active oviposition may be as long as 7 months.

TABLE 4.—*Egg-production data for 4 laboratory-reared Stagnicola bulimoides techella, hatched May 30, 1940*

Snail No.	Date of beginning of oviposition	Number of eggs deposited during—							Total eggs	Date of death	Age	Period of oviposition	Mean daily eggs	Total egg masses	Mean daily egg masses	Mean eggs per mass
		June	July	August	September	October	November	December								
									Number		*Days*	*Days*	*Number*	*Number*	*Number*	*Number*
1	June 17	274	1,295	1,056	2,942	912	----	----	6,479	Oct. 31	152	129	50.2	303	2.3	21.5
3	June 15	147	850	1,561	716	----	----	----	3,274	Sept. 17	109	94	34.8	202	2.1	16.3
4	June 14	176	955	1,424	1,917	288	545	461	5,766	Jan. 10	226	211	27.3	414	2.0	13.9
6	June 17	214	2,308	2,099	309	----	----	----	4,930	Sept. 9	100	84	58.7	258	3.1	19.1
Total or average [1]		811	5,408	6,140	5,884	1,200	545	461	20,449			518	39.5	1,177	2.3	17.4

[1] Weighted average.

EFFECT, ON SURVIVAL, OF DESICCATION AND UNFAVORABLE TEMPERATURES

Since temporary pools are the principal habitats of *Stagnicola bulimoides techella* in the Gulf coast prairie of Texas, drought constitutes the most important single factor operating against these snails. Whenever evaporation exceeds precipitation sufficiently long, as it normally does during the summer (fig. 1), the shallow, temporary pools slowly become dry, leaving the snails exposed to desiccation. No inclination is shown by the snails to follow the receding water, but protection against drying is sought by burrowing into the wet mud where they remain until the pool is restored by rains.

To determine the effect of drought on the survival of the snails, collections were made on June 26 and September 27, 1940—about 2 and 5 months, respectively, after the pool had gone dry—from a habitat where a large population had existed. The snails were collected from two adjacent plots, each 1 m. square and approximately 6 cm. deep. The soil was removed in definite layers, kept separate, and washed through a series of fine screens to determine the vertical distribution of the snails and their survival. The results are shown in table 5. The numbers of specimens lying on the surface and in the first centimeter of soil were about equal in both plots. Those lying on the top were dead, whereas more than 10 percent of those found in the loose, upper soil were alive despite its dryness and high temperature. Although fewer snails were found in the lower strata, more of them were alive. The lethal effects of drought in the habitat appear to be immediate rather than cumulative, since the number of snails surviving in the soil at the end of the summer was about equal to that present shortly after the pool had dried. The data show, therefore, that the snails, having once entered the heavy, clay soil,

TABLE 5.—*Vertical distribution and survival of Stagnicola bulimoides techella in the soil of a natural habitat during drought*

PLOT 1.—COLLECTIONS MADE JUNE 26, 1940 [1]

Soil stratum No.	Thickness of stratum	Temperature	Snails		
			Present	Alive	
	Centimeters	*°F.*	*Number*	*Number*	*Percent*
1	[2] 0	146.3	34	0	0
2	1.0	105.8	36	4	11.1
3	2.5	99.5	4	2	50
4	2.5	([3])	0	0	0
Total			74	6	8.1

PLOT 2.—COLLECTIONS MADE SEPTEMBER 27, 1940 [4]

1	[2] 0	100.4	31	0	0
2	1.0	86	25	3	12.0
3	2.5	81.5	14	4	28.5
4	2.5	([3])	2	1	50.0
Total			72	8	11.1

[1] 60 days after pool had gone dry.
[2] Surface of soil.
[3] No record made, but temperature would be 2° to 3° lower than for stratum 3.
[4] 150 days after pool had gone dry.

are capable of withstanding at least 5 months of drought and summer temperatures of at least 105° F. However, the soil may be wet from time to time by sporadic summer rains, some of which may be very heavy.

Adult snails are capable of withstanding long periods of desiccation when protected from the direct rays of the sun. Specimens collected from pools in the field and kept in aquaria for 3 weeks were then placed in a dry, open dish in an unheated room, where they lived from March 19 to June 25, a period of 98 days. Another lot of large snails collected from the surface of drying soil, the orifices of which were filled with mud, was exposed to desiccation under the same conditions as those mentioned above. These snails lived from March 15 to August 20, a period of 158 days.

Although freezing temperatures occur in the Gulf coast prairie, they are of short duration and usually not severe, the lowest on record at the Texas Agricultural Experiment Station, Substation 3, Angleton, Tex., being 10° F. *S. bulimoides techella* continued to be active during this period, and no evidence of death was observed as a result of low temperatures. To determine further the ability of the snails to withstand low temperatures, adult specimens were put in a finger bowl containing water, placed in the ice box, and kept there for 33 days except for short periods, when they were taken out to ascertain whether they were alive. All the time that the snails were in the ice box, the water in the dish was covered with ice. No deaths occurred until the water in the dish was allowed to freeze solid, when most of the snails succumbed within a few hours; none survived more than a day.

Since the eggs of *S. bulimoides techella* are deposited on the moist mud at the water's edge or in shallow water, they are exposed to desiccation as soon as the pools begin to dry in the spring. That the eggs do not survive the summer drought is shown by the results of collections made in a pool immediately after a drought of 5 months' duration (table 3). Four days after the snails had emerged from the soil, 98.4 percent were juveniles. Of 942 snails collected, the smallest individuals were 1.5 mm. long, whereas the length of newly hatched snails is only 0.6 mm. Two weeks later, only 37.5 percent of the population were juveniles. Furthermore, the survival of eggs subjected to the lethal action of the sun's rays for 5 months and to soil temperatures of as high as 146.3° F., is unlikely.

Estivation is not limited to drought conditions only but may occur in pools containing water when the temperature becomes unfavorably high. In one semipermanent habitat where large numbers of snails were observed, none could be found 2 weeks later when the temperature of the saturated mud and shallow water reached 100.4° and 102.2° F., respectively. After a rain and lowered temperatures, the original population reappeared in its former proportion of adults and juveniles.

DISSEMINATION

No evidence of lateral migration by *Stagnicola bulimoides techella* has been obtained. As already stated, these snails do not follow the receding water in the habitats, and so accumulate in large numbers in the remnants of the pools. Lateral movements do occur, however, but they are passive on the part of the snails. Usually they

take place during the runoff of high water resulting from the heavy and prolonged rainfall that frequently inundates the country. When the habitats are covered with deep water, the snails often rise to the top, where they cling inverted to the under side of the surface film of the water. The gentle flow of the water through natural drainage and the movements of wind disseminate them over wide areas.

The main cross-country drainage ditches constitute another means of disseminating the snails. Well overflows that empty into these drainage ditches form pools where the snails breed throughout the year. These places become the sources of infestation for pastures below and the drainage ditches become the avenues of distribution whenever sufficient rain occurs to produce a flow of water in them. Likewise, drainage ditches, the bottoms of which are improperly aligned, catch and retain water and form favorable habitats for the snails from which they may be carried downstream.

NATURAL INFECTION WITH THE LIVER FLUKE

Natural infection of *Stagnicola bulimoides techella* with *Fasciola hepatica* was found to occur throughout the year. Of 16,276 snails dissected during 1941 from pastures where infected cattle ranged, 83, or 0.0051 percent, were found to be infected. The highest rate of infection occurred in the fall and winter of 1941, which followed a very wet summer. The monthly rate of infection ranged from 0.07 percent in May to 6.2 percent in November. In one temporary pool where conditions for snail growth and parasitism were ideal, the rate of infection was 2.9 percent on October 28 and 6.6 percent 1 month later, whereas only 0.32 and 1.6 percent were infected on December 19 and January 14, 1942, respectively. No infection was found in snails in an adjacent semipermanent habitat (fig. 2. *A*) that harbored a large population of snails and that was accessible to the same cattle as the temporary pool. Sinitsin (*15*) reported 4 percent of *Stagnicola bulimoides techella* in the vicinity of Houston, Tex., to be infected with the liver fluke. He did not state, however, whether this involved the general population or a restricted one.

SUMMARY

Before effective measures against the liver fluke of cattle and sheep can be formulated, a knowledge of the bionomics of the lymnaeid snail that serves as its intermediate host is essential. A study of the literature shows that the bionomics of the various lymnaeid snails that serve as the intermediate hosts of the liver fluke throughout the world varies according to the species involved and the conditions under which they live.

The Gulf coast prairie of Texas, which is in the upper part of the Gulf coast region of that State, is a uniformly flat and low area with a mild, moist climate, and heavy, clay soils that are poorly drained. These conditions provide a favorable habitat for *Stagnicola bulimoides techella*, the common intermediate host of the liver fluke in the United States. Consequently, field and laboratory investigations in this area were made in 1940 and 1941 to study the principal types of habitats, the life history of the snails, their reproduction, dissemination, and related subjects.

The study showed that the snails live in the temporary and semi-permanent pools that are scattered over the entire area. The pools are generally only slightly alkaline in reaction and have extensive soil-water peripheries where the snails live.

The life cycle of *S. bulimoides techella* follows a rather well-defined pattern with two generations annually. One generation occurs in midwinter and the other late in the spring. When the habitats become dry in the spring or early in the summer, the young snails estivate in the soil until the fall rains. Development of the snails is rapid under favorable conditions, being as short as 20 days from egg to egg. The adult snails usually die when about 6 months old, after the period of reproduction.

Under laboratory conditions, the snails were found to remain alive as long as 25.5 months, during which time standing water was present in the aquarium for 2 to 7 days only at the beginning of each month. The snails are capable of withstanding periods of desiccation as long as 5 months under field conditions when estivating in the soil or when exposed in an open dish in the laboratory. The lethal effect of drought on the snails when the pools become dry is immediate rather than cumulative.

Egg production of individual snails is high. They have been found to produce as many as 6,479 eggs during a life span of 5 months and a laying period of 129 days. The snails surviving the summer drought are predominantly juveniles; they emerge from estivation when the fall rains restore the pools, develop rapidly, and oviposit. The snails do not migrate, but they may be distributed widely by water during periods of heavy rainfall when the country is inundated.

The mean infection with liver fluke of 16,276 snails dissected during one year was 0.0051 percent. In one temporary pool the rate of infection in the latter part of November was 6.6 percent.

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A STUDY OF DORMANCY AND GERMINATION OF SEEDS OF CERCIS CANADENSIS [1]

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ANATOMY OF THE SEED

The seed of redbud (*Cercis canadensis* L.) is of the albuminous type. It has a very thin but strong seed coat, a considerable amount of perisperm and endosperm tissue, and, at the time of maturity, a well-developed embryo (fig. 1).

The seed coat (*a*), ranging in color from light tan to dark brown, is composed of small, thick-walled cells impermeable to water. These cells are cylindrical in form with the long axis perpendicular to the surface of the seed, and are commonly referred to as Malpighian cells. The perisperm (*b*), found immediately under the seed coat, is composed of fairly small, thick-walled cells. Unlike those of the endosperm, these cells are arranged in more or less regular rows, both radially and tangentially. The endosperm cells are thin-walled and irregular in shape and arrangement (*c*). The presence of a distinct perisperm in tree seeds is rather unusual, this tissue being normally either entirely absent or so similar in structure to the endosperm that it can hardly be differentiated from it. The mature embryo (*d*), occupying the central cavity of the endosperm, is about 4 mm. long with cotyledons 2½ to 3 mm. wide. In a ripe seed it is fully developed, and all its parts are completely differentiated. The seed is oval in form, somewhat flattened, and is borne in a pod varying in length from 6 to 8 cm.

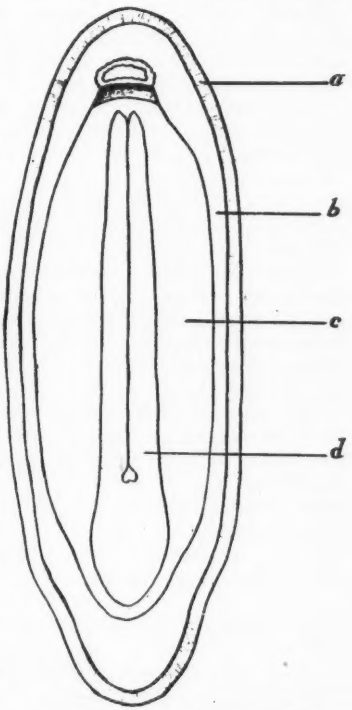

FIGURE 1.—Longitudinal section of redbud seed: *a*, Seed coat; *b*, perisperm; *c*, endosperm; *d*, embryo.

In Oklahoma the seed ripens at the end of August or the beginning of September, but remains on the tree throughout the winter. While still on the tree the seeds are subject to injury and destruction by

[1] Received for publication May 25, 1943. The work covered in this paper is part of an investigation carried on during the previous 4 years. (Bankhead-Jones research project) at the Oklahoma Agricultural Experiment Station, at Stillwater, Okla.

weevils. When collected in the late fall or winter the seeds were invariably worthless, being heavily infested with insects. In nature the seeds which escape injury and fall to the ground usually remain dormant for several years.

MATERIALS AND METHODS

Seeds of the redbud used in this investigation were either collected in Oklahoma (lots 39–0, 39–B, 39–C, 40, 41), mostly in the vicinity of Stillwater, or purchased from a commercial seed dealer in Boston, Mass. (38–A, 38–B). A few experiments, chiefly for the verification of the original results, were performed with seeds collected in Kansas (39–K). With the exception of a few small lots collected between June 28 and August 25 of 1939 and used for the specific purpose of determining some properties of immature seeds, all seeds were collected when completely ripe.

Seeds after being collected and air-dried for several days were stored in glass or metal containers, usually at a temperature ranging between 35° and 50° F. Germination tests, as a rule, were carried out on moist cotton in Petri dishes. However, in some instances indicated in the text, the seeds were tested also in soil, either in flats or in the nursery seedbeds; and in a few cases, tests were made in Erlenmeyer flasks as described elsewhere in this paper. Only sound seeds were used in all tests. These were easily separated from the decayed, empty, or otherwise poor seeds after a treatment with concentrated sulphuric acid followed by washing and soaking seeds in cold water. Poor seeds failed to absorb water in large quantities and usually remained thin and shriveled, while sound seeds imbibed water freely and swelled to several times their original volume in 8 to 12 hours.

The stratification medium used throughout the investigation was granulated peat moss purchased from a chemical company in Kansas City, Mo. When storage at low temperatures was needed, the samples were held either at 37° to 45° F. in an electric refrigerator or in a cold room in which the temperature varied irregularly from 35° to 53° F. with an average of 44°.

It is estimated that more than 50,000 seeds of redbud were used during the investigation.

CAUSES OF DORMANCY AND SEED TREATMENTS

CAUSES OF DORMANCY

Two factors are responsible for delayed germination of redbud seed. One is restriction of the intake of water caused by the nature of the seed coat, a characteristic very common in seeds of legumes (*6, 9*).[2] Another lies in the embryo, which fails to grow even when the seed absorbs a large quantity of water. Several samples of sound fully swollen redbud seeds remained under germinative conditions in the writer's laboratory for as long as 716 days without germinating. Dormancy of the embryo is responsible for the failure of fully swollen seed to germinate. Excised embryos kept under conditions favoring growth usually exhibited phototropism and geotropism,

[2] Italic numbers in parentheses refer to Literature Cited, p. 420.

produced chlorophyll, but failed to grow and produce seedlings. Only in a few instances have excised embryos been observed to grow to a considerable size (*2*). From the practical point of view, redbud embryos should be considered dormant and the seed treated accordingly.

SEED TREATMENTS

Two separate problems are involved in forcing the germination of redbud seed: (1) Modification of the seed coat in order to permit absorption of moisture which is necessary for afterripening; and (2) afterripening of the seed. The treatment of redbud seeds prior to germination does not differ from that of other seeds having similar causes of delayed germination.

The impermeable character of the seed coat can be modified by any of the three methods commonly used on the so-called "hard" seeds in commercial and experimental work (*8, 12, 13*). The standard treatment of seeds with concentrated sulfuric acid (sp. gr. 1,842) proved to be particularly effective, and once the requirement for any one lot had been determined, acid treatment was used throughout the period of investigation whenever the seed coats had to be made permeable to water. As would be expected, individual lots of seeds varied somewhat in the intensity of acid treatment required. No attempt was made to standardize the temperature at which the treatments were carried out. The temperature varied between 70° and 85° F.

At the beginning of the investigation, the optimum period of acid treatment was determined by germination tests of seeds treated for various periods. Later, however, the ability of treated seed to absorb water and the freedom from injury of treated seed were used as the criteria of the proper length of the treatment. Undertreated seeds when placed in water do not absorb it and their volume remains unchanged. Overtreated seeds, on the other hand, absorb water freely and swell to several times their original volume, but also exhibit definite sign of overtreatment in the form of "burned off," light-colored spots appearing on the seed coat (fig. 2). Correctly treated seeds when placed in water swell markedly in 8 to 12 hours, yet their coats remain smooth and intact. Owing to a certain degree of structural variation in seed coat of the same species, it is impossible to determine a "perfect" treatment for any lot of seeds. Any treatment that will render every seed permeable to water will result in overtreating at least a few of the seeds. Table 1 shows the proportions of swollen and "spotted" (injured) seeds caused by treatment with concentrated sulfuric acid followed by soaking in water.

TABLE 1.—*Proportion of swollen and injured seeds caused by acid treatment of various lengths of time; lot 39–K*

Condition of seeds	Not treated	Acid-treated for—				
		10 minutes	20 minutes	30 minutes	40 minutes	60 minutes
Percent of seeds swollen	0	5	60	100	100	100
Percent of seeds "spotted"	0	5	15	35	85	90

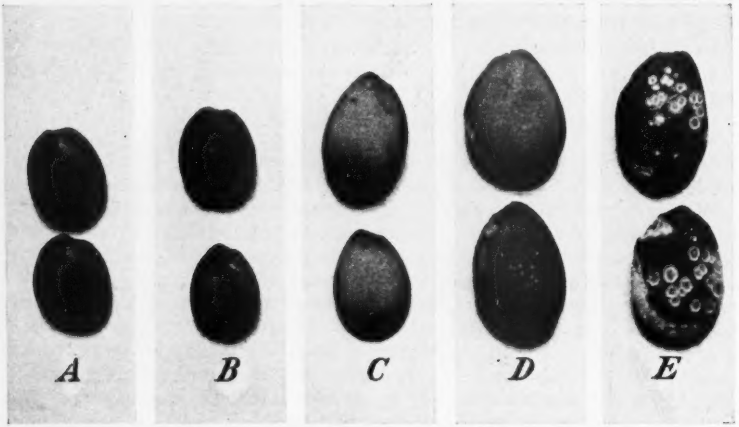

FIGURE 2.—Redbud seeds after various periods of sulfuric acid treatment followed by soaking in cold water for 24 hours (lot 39–0): *A*, Neither treated nor soaked; *B*, not treated but soaked in water; *C*, acid-treated 10 minutes; *D*, acid-treated 20 minutes; *E*, acid-treated 40 minutes.

In determining the optimum period of treatment the question arises as to whether it is more desirable to obtain permeability in all seeds and have some seeds overtreated or to leave part of the seeds with the seed coats still impermeable to water but avoid as much as possible the injury to the seed coats from overtreating. It should be stated that a slight injury to the seed coat does not affect the soundness of the kernel or interfere with the afterripening and germination of the seed, although more serious injury may pave the way for its decay and ultimate destruction. The shortest period of treatment that causes all seeds to absorb water does not usually result in a critical injury to any of the seed and should therefore be considered as the optimum.

The needed length of treatment as determined by the ability of seed to absorb water can be ascertained in a few hours. The optimum treatment of each lot used in this investigation was found by treating small lots of seed for from 10 to 60 minutes. Seeds less than 1 year old responded best to treatment for from 25 to 30 minutes. With the lengthening of the storage period between the time of collection and the time of treatment (age), the response of the seed to acid treatment became slower. The most effective treatment (30 minutes) during the first winter after collection was not sufficiently long when applied to the same lot of seed after a storage of 15 months, as is indicated by the accompanying tabulation:

Lot No.	*Optimum period of acid treatment—minutes*
38–B	30
39–K	25
39–O	30
38–B (after 15 months of dry storage)	45

Hot or boiling water was another medium that was effective in rendering the seed coats permeable to water. Only a small portion of the seeds of lot 38–B soaked in water heated to 180° F. were made

permeable to water, whereas submerging them in boiling water for ¼ minute, ½ minute, 1 minute, and 2 minutes resulted in absorption of water by 62.5, 86.5, 97.5, and 97.5 percent of the seeds, respectively. Seeds of the same lot properly treated with concentrated sulfuric acid (30 minutes) responded 100 percent to the treatment (table 2).

TABLE 2.—*Comparative effectiveness of hot water and sulfuric acid treatments on permeability of seed coat to water, lot 36–B*

Treatment	Percent of seed swollen after 35 days in stratification	Percent germination after indicated days in stratification		
		7	21	35
Boiling water, ¼ minute	62.5	0.5	12.0	28.5
Boiling water, ½ minute	86.5	1.5	26.0	44.0
Boiling water, 1 minute	97.5	2.5	18.0	47.0
Boiling water, 2 minutes	97.5	4.5	6.5	4.5
H_2SO_4, 30 minutes	100.0	4.0	60.5	[1] 83.0
Hot water (180° F.)	Few	0	0	1.0

[1] Stratified 28 days.

Use of boiling water even for 1 or 2 minutes has never given as favorable germination results as did the treatment with concentrated sulfuric acid. Although boiling the seed for ½ minute or less did not sufficiently modify the seed coats in all seeds, boiling for 1 or 2 minutes damaged embryos in at least some of the seeds. Lengthening the period of boiling-water treatment from ¼ to ½ minute or to 1 minute increased the percentage of seeds rendered permeable to water as well as the final percentage of germination. Boiling the seed for 2 minutes reduced germination markedly. Attempts to modify the seed coat by ether, which was found to be effective in increasing the percentage of germination of seeds of *Robinia pseudoacacia* (*9*), proved to be unsuccessful with redbud. Mechanical scarification was highly effective on redbud when each individual seed was scratched, cracked, or sandpapered. No mass treatment of seeds by a mechanical scarifier was tried.

Since the impermeability of the seed coat to water is not the only cause of the delayed germination of redbud seed, modification of seed coat alone is not sufficient to break its dormancy completely: stratification of the seed is also necessary.

Many thousands of seeds of several crops were subjected to stratification during this investigation. The results of seven series of tests (typical for the redbud seed) are presented in table 3. The stratification requirements of individual lots varied considerably, as did those of individual seeds of any one lot. Yet despite these variations two definite conclusions concerning stratification requirements of redbud seeds appear to be justified.

(1) All seeds of redbud must complete afterripening and cannot be forced to germinate unless previously kept under conditions favoring this process. Stratification at 35° to 45° F. favors afterripening.

(2) A stratification period of 5 to 8 weeks at 35° to 45° F. is required for the completion of afterripening of 90 percent or more of seeds in practically all instances. In individual cases, germination of more than 80 percent was secured after less than 4 weeks of stratification (lots 38–B and 39–0) and in one lot (38–B) the seeds germinated to the extent of 94 percent after only 22 days of stratification.

TABLE 3.—*Effect of the length of the stratification period on afterripening of acid-treated [1] seeds of redbud*

Days in stratification	Percent germination in lots—[2]						
	38–A	38–B	38–B_1	38–B_2	39–K	39–O	39–C_1
0					0	0	0
5			4.0	0			
7					15.0	4.0	
8		28.0					
11			4.0	3.0			
14							10.0
15		50.0	4.0	3.0			
21					42.5	60.5	13.0
22		94.0					
25	40.0		10.0	17.0			
28					79.5	83.0	22.0
29	70.8	88.0					
30			14.0	11.0			
35	50.0	96.0	34.0	34.0	97.0		31.0
40			26.0	50.0			
42					99.0		80.0
46		92.0	55.0	89.0			
49							90.0
50			84.0	95.0			
55	47.0	81.0					
56	100.0	88.0			100.0	96.0	
60			88.0	95.0			
63					99.5	95.0	
64							97.0
65			53.0	72.0			
70			23.0	60.0	98.5	94.5	
75			16.0	45.0			
80							
81			56.0	86.0			
84							
86			57.0		99.0	97.0	
91					100.0	99.5	
92							
93			9.0	1.0			
98						100.0	
100				3.0			
105						99.5	

[1] Seeds treated 25 or 30 minutes according to the requirement of individual lots.
[2] The dates in 1939 on which first germination tests were started for the different lots follow: 38–A, Feb. 27 38–B, Mar. 15; 38–B_1, June 27: 38–B_2, June 27; 39–K, Nov. 10; 39–O, Nov. 10; 39–C_1, Oct. 4.

Extension of the stratification period for a few weeks beyond that needed for the completion of afterripening does not seem to interfere seriously with the ability of the seed to germinate. Therefore it appears safe to delay planting of afterripened seed should such delay become necessary. The only two samples of seeds in which germination was reduced with the extension of the stratification period were those stratified on June 22, 1939 (lots 38–B_1 and 38–B_2). Since germination of afterripened redbud seed is adversely affected by extremely high temperature (see table 7), it is very probable that this factor was responsible for the reduction in germination of seeds of these lots rather than the extension of stratification beyond the period 7 to 9 weeks, at which time germination reached its maximum. Germination tests of these two lots were carried on into the late summer when the temperature in the laboratory often exceeded 90° F., whereas the temperature of germination of other lots varied between 70° and 85°.

In addition to the sulfuric acid and stratification method of germinating redbud seeds, various other "forcing" methods and agents were tried. A summary of these treatments and of the results obtained is presented in table 4. For the purpose of comparison, seeds stratified after a treatment with sulfuric acid are also included in the table.

None of the treatments were so effective in afterripening of redbud seed as the combination of proper sulpuric acid treatment and stratification.

TABLE 4.—*Effect of various treatments on afterripening and germination of redbud seeds: lot 38–B*

Treatment	Highest germination	
	Percent	Period in days
None	0	716
H_2SO_4 30 minutes	10.0	365
Stratification 125 days	1.0	90
H_2SO_4 30 minutes and O_2 100 percent for 7 days	0	[1] 7
H_2SO_4 60 minutes, followed by freezing for 21 days	0	[2] 18
H_2SO_4 30 minutes, followed by stratification at weekly alternations of temperature 35°–75°	3.0	50
H_2SO 60 minutes, and concentrated HNO_3, 1 minute	25.0	24
H_2SO_4 15 minutes, followed by stratification for 36 days (41° F.)	46.0	18
H_2SO_4 30 minutes, followed by stratification for 35 days (41° F.)	96.0	8
H^2SO_4 60 minutes, followed by stratification for 36 days (50° F.)	29.0	39
H_2SO_4 60 minutes, followed by stratification for 27 days and soaking in 1 : 5,000 formic acid for 20 hours	38.0	15
H_2SO_4 60 minutes, followed by stratification for 27 days and soaking in 1 : 5,000 NaOH for 20 hours	56.0	15
Boiling water 2 minutes followed by stratification for 35 days (41° F.)	4.5	33

[1] In oxygen.
[2] All dead.

Substitution of freezing for stratification not only failed to bring about afterripening but actually injured the seed. Injury to the embryo caused by mistreatment, mishandling, or by application of certain chemicals always became evident in a few days after the seeds were placed under conditions favoring germination. The loss of viability of injured seeds manifested itself first in a rapid development of fungi on and around the seeds and then in a complete decay and destruction of the affected seeds.

Use of concentrated nitric acid to supplement sulfuric acid treatment caused germination of 25 percent of seeds without stratification but damaged the remainder of the lot. Application of weak solutions of formic acid and sodium hydroxide markedly hastened germination as compared with the application of H_2SO_4 alone, and increased the total percentage of germinated seeds. Germination of seeds not treated with sodium hydroxide was 29 percent as compared to 56 percent for seeds soaked in sodium hydroxide for 20 hours. Increasing the oxygen concentration of the air to 100 percent did not produce as good germination results as stratification, although it had a very pronounced stimulative effect on germination of afterripened seeds, as will be shown later.

An attempt was also made to hasten afteripening in stratification by holding acid-treated seeds in pure oxygen before placing them in stratification. Such treatment not only failed to bring about the desired result but actually lowered germination. After 6 weeks of stratification, 98 percent of seeds not treated with oxygen had germinated while seeds held for 24 hours in pure oxygen prior to stratification germinated to the extent of only 36.5 percent (table 5). A similar effect of the increased oxygen supply on afterripening has been observed in the case of red cedar seeds (*3*, *10*).

TABLE 5.—*Effect of vitamin B_1 and pure oxygen on afterripening*

Treatment	Percent germination after stratification for—		
	2 weeks	4 weeks	6 weeks
H_2SO_4, 30 minutes; vitamin B^1, 24 hours; stratification	18.0	38.5	99.5
H_2SO_4, 30 minutes; pure oxygen, 24 hours; stratification	2.0	6.5	36.5
H_2SO_4, 30 minutes; soaking in water, 24 hours; stratification	15.5	27.5	98.0

In view of frequent claims of the extraordinary stimulative effects of vitamin B_1 on plant growth, a few tests to obtain information as to its value were carried out. One of these dealt with the effect of the vitamin on afterripening. Seeds of lot 39–C, after being treated for 30 minutes with concentrated sulfuric acid, were soaked for 24 hours in a solution containing 4 mg. of vitamin B_1 in a liter of water. After the completion of the treatment the seeds were stratified and the rate and degree of afterripening were compared with those of seeds which were treated with sulfuric acid and then stratified without being soaked in the vitamin solution. In each test germination of vitamin-treated seeds was slightly higher than that of untreated seeds (table 5). The differences in total germination, however, were too small to be significant.

MICROCHEMICAL TESTS

Observations on stored food and three oxidizing enzymes in the seed were made by means of the following tests and reagents: Protein-biuret reaction, fats-Sudan IV; reducing sugars-Flückiger reaction; starch-iodine potassium iodide solution; oxidase-alcoholic solution of gum guaiac; peroxidase-alcoholic solution of gum guaiac and 3-percent solution of hydrogen peroxide; catalase-3-percent solution of hydrogen peroxide neutralized with calcium carbonate.

Afterripening of redbud seed is accompanied by or consists of a number of chemical and physical changes within the seed, more or less like those found in other seeds (*1*, *3*, *5*, *10*). Microchemical tests made on dormant redbud seed revealed large quantities of protein and fats, both in the endosperm and the embryo. Protein was particularly abundant in the cotyledons. Fats were abundant in the tips of the cotyledons and in the endosperm, yet present also in the radicle and the hypocotyl. Neither starch nor sugars were detected in a dormant seed. Oxidase was absent or inactive but the presence of peroxidase and catalase [3] was easily detected.

The embryo of a dormant seed is slightly acid (pH 6.7). The moisture content of an air-dry dormant seed including the seed coat is equal to approximately 10–10.5 percent of the fresh weight of the seed.

The microchemical tests at various stages of afterripening and during germination were made on seeds after 2, 4, 6, and 8 weeks of stratification preceded by treatment with concentrated sulfuric acid. Further statements pertaining to the changes in the amounts of various substances in seeds are based on the changes in the intensity of the re-

[3] Changes in catalase activity were determined quantitatively and are discussed later.

actions brought about by the application of proper reagents. A summary of the changes resulting from afterripening and germination is presented in table 6.

TABLE 6.—*Summary of various changes within the redbud seed as a result of afterripening and germination*

[Differences in number of crosses indicate relative changes in the amount of individual substances]

State of seed	Proteins	Fats	Starch	Reducing sugars	Oxidase	Peroxidase	Catalase [2]	pH	Moisture content (percent fresh weight)
Dormant [1]	XXX	XX	None	None	X	None	11.25	6.7	51.0
Stratified 2 weeks	XXX	XX	None	None	X	X	13.5	.....	55.6
Stratified 4 weeks	XXX	X	None	Trace	X	X	17.0	6.3	55.8
Stratified 6 weeks	XXX	X	None	Trace	X	X	18.0	6.2	57.4
Seed germinated in stratification after 8 weeks	XX	X	XXX	X	XX	XX	23.4	6.5	63.5

[1] After sulphuric acid treatment and 24 hours of soaking.
[2] Cubic centimeters of O_2 evolved from 5 cc. of H_2O_2 in 10 minutes per 0.1 gm. of dry material.

As afterripening progressed the amount of proteins and fats decreased markedly, particularly the latter. However, after 6 weeks of stratification there still was a fair quantity of both in the endosperm and much protein in the embryo. Fats were still present in cotyledons and in the radicle but had disappeared from the hypocotyl.

The presence of reducing sugars was detected in seeds after 4 weeks of stratification. At that time sugars appeared in the cotyledons and in the radicle but were absent from the endosperm. The first appearance of reducing sugars in the endosperm was noted after 6 weeks of stratification, and even then only in very small amounts in the area adjacent to the embryo.

After 2 weeks of stratification oxidase appeared in the cotyledons, along their inner edges. After 4 weeks it became active throughout both the endosperm and the embryo. Peroxidase and catalase increased continuously throughout the period of afterripening. The reaction of the kernel changed from the original pH of 6.7 in a dormant seed to 6.3, 6.2, and 6.5 after 4, 6, and 8 weeks, respectively, of stratification. As a result of sulfuric acid treatment and soaking overnight, the moisture content of the seed increased from 10.3 percent to 32.6 percent of the weight of fresh material. Further soaking of the seed at room temperature (for a total of 24 hours) raised the water content of the seed to 51.0 percent. During 6 weeks of stratification, moisture in the seed remained more or less constant, fluctuating between 54.3 and 57.4 percent.

GERMINATION

MICROCHEMICAL CHANGES

Germination is accompanied by a sharp increase in moisture content of the seed, further reduction in the amount of proteins and fats, increase in the amount of reducing sugars and in the activity of oxidase, peroxidase, and catalase, a slight reduction in the acidity of the embryo, and a sudden appearance of a large quantity of starch (table 6). Starch is abundant in the cotyledons, the outer part of the hypocotyl,

and in the tip of the radicle, although some appears also in the endosperm.

TEMPERATURE

Germination of afterripened redbud seed takes place at a rather wide range of temperatures, from 33° to 100° F., and probably even higher. Tests to determine the range of germination temperature and the optimum temperature were conducted on a total of 3,200 seeds, one-half of which, after being treated with sulfuric acid for 30 minutes, were stratified at 41° F. for 6 weeks and the other half for 8 weeks. Table 7 presents a summary of the progress of germination of 16 samples of seed (200 each) placed and kept in germinators at various temperatures, from 33° to 100° F.

TABLE 7.—*Germination of redbud seeds as affected by temperature*

Germination temperature (° F.)	Percentage germinations [1] after—			
	2 days	4 days	6 days	8 days
33°	0	0	0.7	0.7
42°	.25	.25	1.7	1.7
48°	.50	3.75	15.5	24.0
60°	25.2	55.7	84.2	91.0
70°	72.2	91.2	96.5	96.5
80°	87.5	90.7	91.2	91.2
90°	90.5	92.0	93.0	93.0
100°	29.0	45.5	50.5	53.7

[1] Average of 2 samples, one stratified for 6 weeks, the other for 8 weeks.

Within the range of 33° to 90° F. the rate of germination was directly affected by the temperature. Two days after the seeds had been set in germinators, 90.5 percent germinated at 90° while germination at 48° and below was less than 1 percent. After 4 days, germination of more than 90 percent was obtained at 70°, 80°, and 90°, and after 8 days more than 90 percent of the seeds germinated also at 60°. At 100° germination rose from 29.0 percent in 2 days to 53.7 percent in 8 days. The total germination (in 8 days) was highest at 70° (96.5 percent), although at that temperature it proceeded more slowly than at 80° or 90°. The temperature of 70° appears to be the optimum for germination of redbud seed. It happens to be the most favorable temperature also for growth of small seedlings. At a temperature of 60° and below seedlings grew more slowly than at 70°, although they apparently remained normal and sound. At a temperature of 80° and above, a certain degree of deterioration was evident among the seedlings. This was particularly evident at 100° at which temperature many of the seedlings rotted and others assumed an unhealthy abnormal appearance.

OXYGEN

Although afterripened redbud seeds germinate freely and rapidly in the normal atmosphere, and even under water when air is bubbled through it, an increase in the oxygen content of the atmosphere or the addition of it to water had an exceptionally stimulative effect on germination. Whereas germination of well afterripened seeds under normal conditions may be expected to be completed in a period of

6 to 8 days, afterripened seeds kept in pure oxygen were observed to germinate to the extent of 100 percent in 2 days.

In the investigation of the effect of oxygen on germination, a sample of seeds was placed in a cheesecloth bag suspended in a flask filled with pure oxygen. A few cubic centimeters of water were left at the bottom of the flask to prevent drying of the seeds and to provide the necessary moisture for germination. Another sample was placed in 100 cc. of distilled water through which oxygen was bubbled at the rate of 20 bubbles per minute. Checks for these treatments were a standard germination set-up (Petri dishes with moist cotton) and a sample of seeds placed under water. The original intention was to keep the seeds under treatment for 2 days and then to transfer them to the normal conditions of germination for comparison of their behavior with that of normally handled seeds. However, at the end of the first day, 1.5 percent of the seeds serving as a check in a Petri dish had germinated, while germination of seeds held in pure oxygen was 71.5 percent. At this time the seeds were removed from the oxygen flask and placed in moist cotton in a Petri dish in normal atmosphere. One day after this transfer germination of the oxygen-treated seeds rose to 90 percent while that of untreated seeds was only 13.5 percent. In a similar experiment carried out prior to the one just discussed, afterripened seeds kept in pure oxygen for 48 hours germinated 100 percent during that period as compared with 18 percent germination of seeds held in the normal atmosphere (table 8).

TABLE 8.—*Germination of afterripened seed under the influence of variations in the oxygen content of air and water*

Treatment	Percentage germination [1] after—				
	1 day	2 days	3 days	4 days	5 days
48 hours in 100 percent oxygen	10	100.0			
Check (normal atmosphere)		18.0			
24 hours in 100 percent oxygen	71.5	90.0		96.5	97.5
Check (normal atmosphere)	1.5	13.5		90.5	94.0
Set in nonaerated water	0	0	0	0	0
Set in water with O_2 bubbling through		92.0			

[1] Days from the time of setting of the experiment.

Bubbling oxygen through water in which afterripened seeds were placed had nearly the same effect as keeping the seeds in pure oxygen. Under these conditions germination of 92.0 percent was secured in 2 days. No germination took place among the seeds kept continuously under nonaerated water.

VITAMIN B_1

While treatment of nonafterripened seed with vitamin B_1 might have had a slight stimulative effect on the process of afterripening, the vitamin applied to afterripened seed was found to be entirely ineffective in stimulating germination. Afterripened seeds treated for 24 hours with a vitamin B_1 solution of 4 mg. per liter germinated almost at the same rate and to the same extent (95.5 percent) as the seeds that were not treated (97.5 percent).

EFFECT OF DRYING ON AFTERRIPENED SEEDS

During a normal procedure of afterripening, planting, or other handling of seeds, there is always a chance for them to dry more or less. Accidental drying of afterripened seeds may in some instances reduce germinability, throw seeds into a state of secondary dormancy (*3*), or even destroy their value completely by reducing their viability (*7*). Knowledge of the effect of drying on afterripened seeds seems to be of practical importance, suggesting the degree of care to be exercised while handling, storing, or shipping them.

Sixteen hundred seeds treated 30 minutes with sulfuric acid and kept in stratification for 55 days were placed in the laboratory at 75° to 85° F. and allowed to dry. From time to time samples of the seeds were tested for their ability to germinate. The results, presented in table 9, show that drying for 6 days had no effect on the continued ability of an afterripened embryo to resume growth. After 10 days of drying, germination fell off from the original of 96 percent to 90 percent, but when dry storage was extended to 30 days germination was reduced to 78 percent. Further extension of the dry storage to 60 and 90 days lowered germination to 4 and 6 percent, respectively.

TABLE 9.—*Effect of drying of afterripened seeds* [1] *on their ability to germinate*

Period of dry storage (days)	Percent germination	Moisture content after drying (percent of total weight)	Period of dry storage (days)	Percent germination	Moisture content after drying (percent of total weight)
0 (check)	96	56.4	10	90	10.2
1	98	6.2	30	78	11.7
2	94	6.9	60	4	11.3
4	92	7.7	90	6	
6	98	9.0			

[1] All seeds treated 30 minutes with concentrated sufluric acid, then stratified 55 days.

A sharp decline in the germination ability of afterripened seeds kept dry for 60 and 90 days was not caused by an additional loss of moisture Under conditions of dry storage (temperature 75° to 85° F.) moisture equilibrium in seeds was reached after 24 hours of drying. Continued exposure of seeds to a temperature of 75° to 85° beyond that period resulted in an increase in moisture content of the seed which was probably due to the increase in the humidity of the surrounding atmosphere.

Drying afterripened seeds in an oven affected the viability of the seeds in various degrees, depending not only on the temperature of drying but also on the moisture content of the seeds at the time when the seeds were placed in the oven. When partially dried, redbud seeds were able to withstand considerable heat. In this investigation, seeds dried at room temperature for 4 days with moisture content reduced to 7.7 percent of the total weight of the seeds lost in viability from the original of 96 percent to 81 percent after being exposed to a temperature of 167° for 2 days.

The ability of partially dried seeds to withstand abnormally high temperatures is not uncommon. The present technique of seed extraction of coniferous species involves the use of dry kilns in which the temperature is raised to 110° and 120° F. (*14*).

CATALASE ACTIVITY

Catalase determinations in redbud seed were made by the method described by Davis (*4*) and adopted since by many other workers for measuring the activity of this enzyme in plant tissues. During the present investigation the entire seeds rather than kernels were tested for catalase activity because it is extremely difficult, if at all possible, to separate the seed coat from the perisperm. The amounts of various substances used in each catalase test were as follows: Water, 12 cc.; 3-percent solution of hydrogen peroxide, 5 cc.; plant material (seeds), approximately 600 mg.; $CaCO_3$ to equal the weight of plant material; and a pinch of pure quartz sand. In the following discussion catalase activity is expressed in terms of the number of cubic centimeters of oxygen evolved from 5 cc. of hydrogen peroxide in 10 minutes per 0.1 gm. of dry material of the seeds.

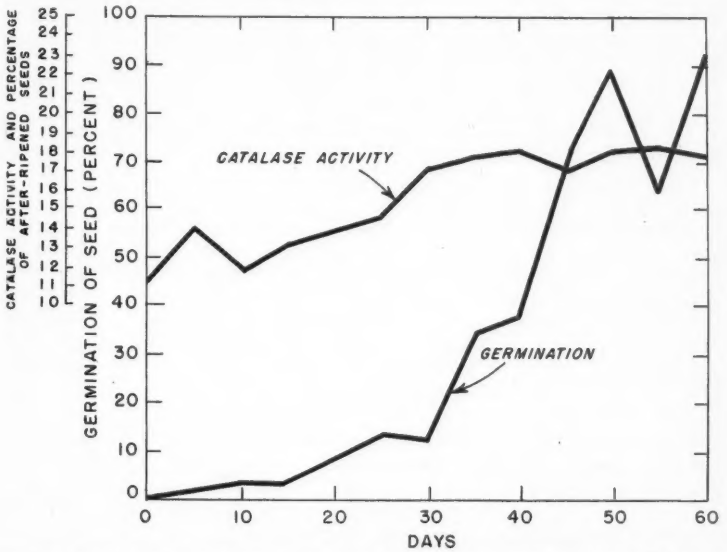

FIGURE 3.—Relation between degree of afterripening and catalase activity in redbud seed. Catalase activity is expressed in terms of cubic centimeters of oxygen given off in 10 minutes by 0.01 gm. of dry material.

Catalase activity was determined in dormant dry seeds, seeds treated with concentrated sulfuric acid and soaked overnight, and seeds kept in stratification for various periods of time under conditions favoring afterripening. To obtain a good representative sample of the entire lot, 75 seeds were used in each test. On the days on which the tests were run, samples of seeds were placed in germinators to determine the degree of afterripening and the readiness of the seeds to germinate. The results of these tests are given in table 10 and figure 3.

Catalase activity in stratified redbud seeds increased with afterripening from 11.25 in dormant seed to an average of 17.95 in seeds more than 90 percent of which had completed their afterripening.

TABLE 10.—*Catalase activity and afterripening during stratification* [1]*; lot 38–B*

Period of stratification (days)	Lot 38–B_2		Lot 38–B_1	
	Catalase [2]	Germination	Catalase [2]	Germination
0	11.25	0	11.25	0
5	13.26	0	14.85	4.0
11	11.20	3.0	12.35	4.0
15	13.50	3.0	13.00	4.0
20	12.80		14.90	
25	15.00	17.0	14.30	10.0
30	17.00	11.0	17.30	14.0
35	18.05	34.0	17.55	34.0
40	18.00	50.0	18.36	26.0
46	17.50	89.0	16.70	55.0
50	18.60	95.0	17.65	84.0
55	18.85	81.0	17.50	47.0
60	18.25	95.0	17.65	88.0

[1] All seeds treated for 30 minutes with concentrated H_2SO_4.
[2] Catalase activity expressed in terms of the number of cubic centimeters of oxygen evolved in 10 minutes by 0.1 gra. of dry material.

The increase was very gradual, not entirely uniform, and not strongly correlated with the increase in germination. This suggests one of two possibilities: either catalase activity is more or less independent of the progress of afterripening, its intensity being simply controlled by the same external factors and in the same direction (though at

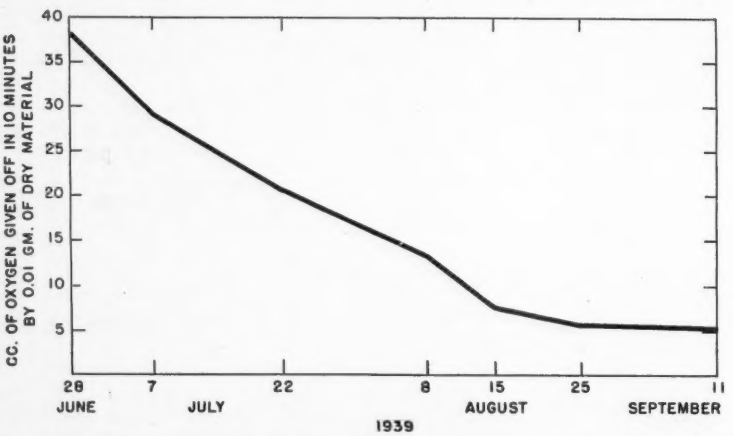

FIGURE 4.—Catalase activity as affected by the maturity of redbud seed. At the time of the two last tests (August 25 and September 11) the seeds were ripe.

different rates) as the afterripening; or, if changes of catalase activity are a part of the afterripening, they precede some other changes that are necessary for the completion of afterripening. The writer is inclined to consider the changes in catalase activity as a part of afterripening because of the close interrelationship between this and other processes on which the progress and the completion of the afterripening depend. Whether the relationship between catalase activity and the progress of other phases of afterripening is constant under all conditions and for all lots of seeds is, of course, open to question. Considerably more work is needed along this line.

In the course of the investigation catalase activity during the ripening process was also studied. Samples of seeds from a single plant were collected and catalase determinations made on various dates from June 28, 1939, to September 11 of the same year. On the former date the seeds were small, green, and soft. The two last tests (August 25 and September 11) were made on seeds which appeared to be completely ripe. The assumption that the seeds were ripe during the last two tests is substantiated by the results of catalase determinations, which showed that catalase activity attained a certain degree of stability.

As was expected (*11*), catalase activity fell off sharply with the ripening of the seed. At the time the seed reached maturity catalase activity was less than one-seventh of what it was 2 months earlier (fig. 4).

SUMMARY AND CONCLUSIONS

Delayed germination of seed of redbud (*Cercis canadensis* L.) is caused by the impermeability of the seed coat to water and by the dormancy of the embryo.

The seed coat can be rendered permeable to water by any of the standard treatments used with "hard-coated" seeds; namely, soaking of seed in concentrated sulfuric acid, hot water or boiling water bath, and mechanical scarification. The optimum treatment with sulfuric acid was of 30 to 35 minutes' duration

Extension of the storage of ripe dormant (unstratified) seeds to 15 months resulted in the lengthening of the required period of acid treatment from 30 to 45 minutes.

Stratification of acid-treated seed at low temperature (35° to 45° F.) was found to be effective in afterripening. The average period of stratification causing afterripening of not less than 90 percent of seeds varied between 5 and 8 weeks.

Holding afterripened seed in pure oxygen for 24 or 48 hours resulted in a marked increase in the rate of germination.

The optimum germination temperature of afterripened redbud seed was 70° F. Some germination occurred at a temperature as low as 33° F. Germination at 100° F. was slow and the seedlings produced and grown at that temperature either became unhealthy and abnormal in appearance or rotted completely.

Vitamin B_1 had no appreciable effect either on afterripening or germination

Ten days of dry storage of fully afterripened seed at 75° to 85° F. reduced the ability of the seed to germinate from the original of 96 percent to 90 percent.

Catalase activity increased during the progress of afterripening.

Catalase activity decreased steadily during the late stages of ripening of the seed.

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